A SIMPLE LOW COST METHOD FOR SCREENING TRANSGENIC AND NON-TRANSGENIC PLANTS FROM A LARGE POPULATION EITHER IN FIELD OR GREEN HOUSE.

FIELD OF THE INVENTION:

The present invention relates to a process and a resultant product used for the identification of transgenically improved plants along with antibiotic resistant or herbicide resistant traits.

BACKGROUND OF THE INVENTION:

Till date all the commercial transgenic crops comes with some marker gene, mostly antibiotic marker or herbicide marker. Here, we propose to use this marker gene as a useful tool for screening the large scale breeding population for a possible admixture of non-transgenic plants or transgenic segregants. This technique is effective only when the marker gene is co-segregating with the useful gene.

Presently, three techniques are commonly followed by industries / research institutes for large scale screening of transgenic plants depending on the gene introduced:

i) <u>PCR Test</u>

Using this technique, particular size of the transgene DNA fragment is amplified which confirms the presence of gene.

ii) <u>ELISA / Lateral Flow Strip Test.</u>

Using the antibody specific for the protein expressed from the transgenene, and the transgenic event is confirmed.

iii) Bio Assay

Either studying resistance to insects, microorganisms or other abiotic stresses in whole/plant parts.

The first two tests are cost intensive, require sophisticated laboratory facility, require technical expertise to perform and are error prone if proper precautions are not taken. The third test is labour intensive, require large space, basic laboratory infrastructure and some times faces seasonal problem associated to availability of insect / pest particular to some resistance genes or adverse abiotic stress conditions.

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OBJECTS OF THE INVENTION

Our proposed system, which is very low cost, does not require any technical expertise / education, highly accurate and work irrespective of the crop or target gene used. However, the system is effectively only when the marker gene and the gene of interest are tightly linked.

This proposed system primarily works in case of transgenic plants made out of transformation using Agrobacterium with single binary vector having both marker and desired gene in between border sequence because they are transferred and segregating together to the progenies due to tight linkage and no crossing over takes place.

Whereas this system does not work in co transformation either by Agrobacterium or other means where the marker gene and desired gene are in different plasmid and are not tightly linked as well segregated in progenies where the marker gene and desired gene moves to different progenies.

SUMMARY OF THE INVENTION

Before the process here the inventor explains the use of marker gene in transgenic development and its mode of action in general (kanamycin, hygromycin, basta etc.)

The Kanamycin, the aminoglycoside antibiotics which affects the protein synthesis in mitochondria and chloroplast because the ribosome found in these

organelles are susceptible to this antibiotic and that are similar to those found in bacteria. The application of this antibiotic to the plant tissues will show chlorosis caused by lack of chlorophyll synthesis and inhibition of growth.

Transgenic plants with NPT II gene or HPT gene has ability to transfer the phosphate group from ATP to the 3-hydroxyl group of the amino – hexose portion of the substrate, aminoglycoside (Kanamycin or Hygromycin) and detoxifies. In other words it catalyses the phosphorylation of the hydroxyl group in the antibiotic Kanamycin or Hygromycin and become inactive. Unlike Kanamycin, the Hygromycin B kills the sensitive cells more quickly.

Similarly, in case of bar and pat gene, they inactivate the substrate Basta or Phosphinothiricin (ammonium glufosinate) by the acetylation of free amino group of the substrate using Acetyl co A as cofactor and preventing it from binding to the Glutamine synthetase (GS) enzyme which plays essential role in the regulation of nitrogen metabolism and ammonia assimilation. If the plant is not transformed with bar or pat gene, the GS will be inhibited by the substrate and results in accumulation of ammonia and disruption of chloroplast structure that leads to the inhibition of photosynthesis and death of plant cells.

BRIEF DESCRIPTION OF THE DRAWINGS:

A better understanding of the present invention and the enhancement of expression level of the invention will be had from the following figures, given as illustrations.

Figure 1: Shows the susceptible plant in which the leaf turned yellowish.

Figure 2: Shows the leaf is green in colour and remains unchanged even after using the antibiotic / herbicide solution.

Figure 3: Shows the leaf turned brownish indicating the susceptible plant,

Figure 4: Shows the leaf turned yellowish in cotton plant after the spraying of the herbicide solution indicating as the non-transgenic plant.

Figure 5: Shows the Cotton plant where in one leaf is sprayed with the antibiotic / herbicide solution after which the leaf is still green in colour indicating as transgenic plant.

DESCRIPTION OF THE INVENTION:

Basically the inventor explains the use of marker gene in transgenic development and its mode of action in general (kanamycin, hygromycin, basta etc.)

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TRANSGENE SEGREGATION PATTERN IN $T_0 \rightarrow T_1 \rightarrow T_2$ etc.

At T_0 level, since the desired transgene and marker gene is linked together as well in heterozygous state, the screening can be done with all putative transgenic plants in Green House by applying the marker chemical (antibiotics etc.) solution on leaf to further confirming the presence of transgene product. Here before going for any costly method like PCR or ELISA for putative transgenics, one can screen the escapees from in vitro culture.

At T1 level the selfed progenics from T_0 are planted in a row and screened for the transgene by applying marker chemical (antibiotics etc.) and the susceptible plants can be uprooted. The positives from these population are cross checked for the transgene and confirmed the presence of gene and product.

At T_2 level the identified homozygous plants, for transgene by resulting 100% positives from T_1 progenies, is used for backcrossing into particular line. The segregating population from this backcross will be screened by applying marker chemical and cross checking using PCR or other means to confirm our technique.

METHOD FOR PREPARATION OF THE SOLUTION IS AS FOLLOWS:

The antibiotic / herbicide etc. solution is prepared in the range or the formulation includes :

1. Kanamycin: 1.0% to 10%; however works best between 2.0% - 5.0%

2. Hygromycin: 0.1% to 2.0%; however works best between 0.25% - 1.0%

3. Basta: 0.1% to 3.0%; however works best between 0.25% - 2.5%

4. All the above marker chemicals may be dissolved in 0.01% solution of Tween 20 or Tween 80 in water.

APPLICATION IN THE PLANT IS OF DIFFERENT TYPES:

1. Painting the solution on upper surface of the leaf for about 2 to 4 cm area using either cotton swab, cotton buds, tissue paper or similar material thereof and marking the place where it is applied to make observation after 1 to 12 (best on 6^{th} / 7^{th}) days or

2. Spraying the solution on full leaf and marking ir. Or

3. Dipping the leaf in antibiotic solution (eg. Using a wide mouth bottle or similar container thereof). Or

4. Keeping wet cotton / paper swab on leaf surface.

OBSERVATION

Yellowing or the leaf occurs in applied area after 5 to 7 days in the case of susceptible plant and maintain the greening in the case of transgenic plants with specific marker gene. After 1 to 12 (best on 6^{th} / 7^{th}) days the leaf from susceptible plant will get browned or charred and maintains the green condition in transgenic plant.